



Lower skeletal muscle capillarization and VEGF expression in aged vs. young men

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Abstract

Recently, we observed that muscle capillarization, vascular endothelial growth factor (VEGF) protein, and the VEGF mRNA response to acute exercise were lower in aged compared with young women. We hypothesized that similar age-related differences in muscle capillarization and VEGF expression would exist between young and aged men. Skeletal muscle biopsies were obtained from the vastus lateralis before and at 4 h after a submaximal exercise bout for the measurement of morphometry, capillarization, VEGF, KDR, and Flt-1 in seven aged (mean age 65 yr) and eight young (mean age 21 yr) sedentary men. In aged compared with young men, muscle capillary contacts and capillary-to-fiber perimeter exchange index were lower regardless of fiber type. Muscle VEGF mRNA and protein were lower in aged men both at rest and 4 h postexercise. Exercise increased muscle VEGF mRNA and protein and KDR mRNA independent of age group. There were no effects of exercise or age on muscle Flt-1 mRNA or protein or KDR protein. These results confirm that skeletal muscle capillarization and VEGF expression are lower in aged compared with young men.

Lower skeletal muscle capillarization and VEGF expression in aged vs. young men

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Ryan, Nicholas A., Kevin A. Zwetsloot, Lenna M. Westerkamp, Robert C. Hickner, Walter E. Pofahl, and Timothy P. Gavin. Lower skeletal muscle capillarization and VEGF expression in aged vs. young men. *J Appl Physiol* 100: 178–185, 2006. First published September 15, 2005; doi:10.1152/jappphysiol.00827.2005.—Recently, we observed that muscle capillarization, vascular endothelial growth factor (VEGF) protein, and the VEGF mRNA response to acute exercise were lower in aged compared with young women (Croley AN, Zwetsloot KA, Westerkamp LM, Ryan NA, Pendergast aged men, Hickner RC, Pofahl WE, and Gavin TP. *J Appl Physiol* 99: 1875–1882, 2005). We hypothesized that similar age-related differences in muscle capillarization and VEGF expression would exist between young and aged men. Skeletal muscle biopsies were obtained from the vastus lateralis before and at 4 h after a submaximal exercise bout for the measurement of morphometry, capillarization, VEGF, KDR, and Flt-1 in seven aged (mean age 65 yr) and eight young (mean age 21 yr) sedentary men. In aged compared with young men, muscle capillary contacts and capillary-to-fiber perimeter exchange index were lower regardless of fiber type. Muscle VEGF mRNA and protein were lower in aged men both at rest and 4 h postexercise. Exercise increased muscle VEGF mRNA and protein and KDR mRNA independent of age group. There were no effects of exercise or age on muscle Flt-1 mRNA or protein or KDR protein. These results confirm that skeletal muscle capillarization and VEGF expression are lower in aged compared with young men.

KDR; Flt-1; fiber cross-sectional area

IN MEN, AGING LOWERS THE OVERALL number of capillaries surrounding skeletal muscle fibers (5, 8, 27, 30). Recently, we have observed that muscle capillary contacts (CC) surrounding type II, but not type I, fibers were lower in aged compared with young women (6). In normal fit and endurance-trained young and aged men, Proctor et al. (30) reported no difference in muscle capillarization of type I fibers but lower CC of type IIA and IIB fibers of aged compared with young men. However, the findings of Proctor et al. must be viewed with some caution because both sedentary and trained men were included in the statistical analysis. When examining the results of Proctor et al. exclusively from sedentary men (30), muscle CC was 18, 30, and 38% lower surrounding type I, IIA, and IIB fibers, respectively, in aged compared with young men. Thus the effect of aging on muscle fiber type-specific capillarization in sedentary men remains equivocal. Reductions in capillarization may be detrimental in aged muscle because skeletal muscle capillarization is an important determinant of maximal oxygen con-

sumption ($\dot{V}O_{2\max}$), insulin sensitivity, and muscle fiber cross-sectional area (FCSA) (18, 19).

Vascular endothelial growth factor (VEGF) is a predominantly endothelial cell-specific heparin-binding, 45-kDa homodimeric glycoprotein mitogen and is an important regulator of basal skeletal muscle capillarization as well as exercise-induced angiogenesis (1, 36). In women, aging lowers resting skeletal muscle VEGF protein and lowers the exercise-induced increase in VEGF mRNA in women (6). However, aged men may demonstrate greater maintenance of muscle capillarization than aged women (5, 30); therefore, VEGF expression may not be different between young and aged men. Consistent with this, the muscle VEGF mRNA response to acute resistance exercise is similar between young and aged men (21). Accordingly, the present study was designed to investigate whether fiber type-specific muscle capillarization is lower and whether muscle VEGF and VEGF receptor expression are lower at rest or in response to acute aerobic exercise in aged compared with young men.

METHODS

Subjects. Eight sedentary young men (range 19–25 yr) and seven sedentary aged men (range 62–72 yr) volunteered to participate in the study after receiving written and verbal explanations of the content and intent of the study in accordance with the University and Medical Center Institutional Review Board. All subjects were healthy nonsmokers, with no history of cardiopulmonary disease. Subject characteristics are listed in Table 1. Subjects were carefully prescreened to preclude participation by individuals with overt cardiovascular disease. Subjects taking medications for cardiovascular disease were excluded. Sedentary subjects were defined as participating in <1 h of strenuous physical activity per week.

$\dot{V}O_{2\max}$ and body composition. $\dot{V}O_{2\max}$ was measured on an electronically braked cycle ergometer (Lode, Excaliber Sport, Groningen, The Netherlands) by open-circuit spirometry (True Max 2400, Parvo Medics, Salt Lake City, UT). The test began with a 5-min warm-up at 125 W for young men and 50 W for aged men. After the warm-up, the workload was increased 25 W for young men or 20 W for aged men every 2 min until volitional fatigue. Body density was determined via hydrostatic weighing, and body fat percent was determined from body density based on a two-compartment model.

Submaximal exercise and muscle biopsies. At least 1 wk after the $\dot{V}O_{2\max}$ test, subjects completed 45 min of cycle ergometer exercise (25 min of exercise, 5 min of rest, and 20 min of exercise) at 50% of $\dot{V}O_{2\max}$. Before the commencement of exercise and at 4 h postexercise, muscle biopsies were obtained from the vastus lateralis. The resting and postexercise muscle biopsy samples were obtained from

Table 1. *Subject characteristics*

	Young	Aged	P Value
Age, yr	21 (2)	65 (4)	<0.001
Height, cm	179.9 (6.4)	177.0 (5.0)	0.355
Mass, kg	75.9 (16.0)	90.5 (6.7)	0.043
Body fat, %	18.4 (5.4)	24.5 (2.7)	0.018
FFM, kg	61.5 (11.1)	68.3 (4.7)	0.160
$\dot{V}O_{2\text{ max}}$, ml $O_2 \cdot kg^{-1} \cdot min^{-1}$	39.7 (5.1)	23.6 (2.7)	0.003
$\dot{V}O_{2\text{ max}}$, ml $O_2 \cdot kg \text{ FFM}^{-1} \cdot min^{-1}$	48.7 (5.2)	31.3 (2.6)	<0.001
Race (Caucasian/African American)	7/1	7/0	

Values are means (SD) for 8 young and 7 aged men. FFM, fat-free mass; $\dot{V}O_{2\text{ max}}$, maximal oxygen consumption.

alternate legs. Samples were stored at -80°C until analysis. A section of the resting biopsy sample was oriented in an optimum cutting temperature-tragacanth mixture, frozen in liquid nitrogen-cooled isopentane, and stored at -80°C until processing for the measurement of muscle morphometry and capillarization.

RNA isolation and real-time PCR. Approximately 10 mg of muscle were homogenized in TRIzol reagent (Invitrogen, Carlsbad, CA). After addition of chloroform and phase separation, RNA was isolated from the aqueous phase by use of an RNeasy fibrous tissue mini kit (Qiagen, Valencia, CA). RNA was quantified fluorometrically using RiboGreen RNA quantitation kit (Molecular Probes, Eugene, OR), and 150 ng were reverse transcribed into first-strand cDNA using MultiScribe RT in the high-capacity cDNA archive kit [Applied Biosystems (AB), Foster City, CA]. Real-time PCR was conducted on 25 ng of cDNA per reaction in 50- μl reaction volumes using TaqMan Universal PCR Master Mix with commercially available (AB) primer and probe sets for human VEGF (product no. Hs00173626_m1) and human KDR (product no. Hs00176676_m1) by use of FAM/TAMRA-labeled dye on an AB PRISM 7000 sequence detection system instrument and software. The Flt-1 primer and probe set was designed using Primer Express software (AB) that selects primer and probes optimized for use with AB system products (forward primer: 5'-AAGGCAAGAAACCAAGACTAGATAGC-3'; reverse primer: 5'-CCTCAACATCACTCAGACTTTTATCTTC-3'; probe: 5'-AAAGC-TTTGCGAGCTCCGGCTTTC-3'). Real-time PCR was run for 1 cycle (50°C for 2 min, 95°C for 10 min) immediately followed by 40 cycles (95°C for 15 s, 60°C for 1 min). Fluorescence was measured after each of the repeated cycles. RNA samples were normalized to 18S rRNA (eukaryotic 18S PDAR primer-limited VIC/TAMARA, AB, product no. 4310893E) multiplexed during the analysis of each specific gene.

Protein isolation and analysis. A 10-mg portion of the muscle biopsy sample was homogenized with a Dounce homogenizer in RIPA (1X PBS, 1% Igepal, 0.5% sodium deoxycholate, 0.1% SDS with protease inhibitors). Total protein was measured by bicinchoninic acid (Bio-Rad Laboratories, Hercules, CA). Commercial VEGF (from 50 μg of total protein), KDR (from 125 μg of total protein), and Flt-1 (from 50 μg of total protein) ELISA kits were used according to the manufacturer's instructions (R&D Systems, Minneapolis, MN) as previously described (14).

Morphometry, morphology, and immunohistochemistry. Muscle tissue from the resting biopsy was sectioned to a thickness of 10 μm on a cryostat, mounted on slides, and kept at -20°C until fixation. Serial sections were stained for capillaries using the double-stain technique (31) as modified by Porter et al. (29) and for fiber type by use of a myosin ATPase stain (3). The myosin ATPase stain identifies muscle fibers as either type I, IIA, or IIB fibers (3). Despite the fact that human skeletal muscle identified as type IIB by histochemical analysis actually express type IIx (35), we have identified fibers types using the myosin ATPase stain nomenclature used in the original work.

Muscle sections were viewed under a light microscope (Nikon 400), and a digital image was taken of the section (Nikon Coolpix 990) as previously described (13). Capillaries were quantified manually from the digital image on individual fibers. The following indexes were measured (19): 1) the number of capillaries around a fiber (CC), 2) the capillary-to-fiber ratio on an individual-fiber basis (C/F_1), and 3) the number of fibers sharing each capillary [sharing factor (SF)]. Capillary density (CD) was calculated by using the fiber as the reference space. Capillary-to-fiber perimeter exchange index (CFPE) was calculated as an estimate of the capillary-to-fiber surface area. Quantification of the capillary supply was performed on at least 50 fibers by randomly selecting a fiber in an artifact-free region. Fiber cross-sectional area (FCSA) and perimeter (FP) were measured with the image-analysis system and commercial software (SigmaScan, Jandel Scientific), calibrated to transform the number of pixels (viewed on a computer monitor) into micrometers from an image of the myosin ATPase stain.

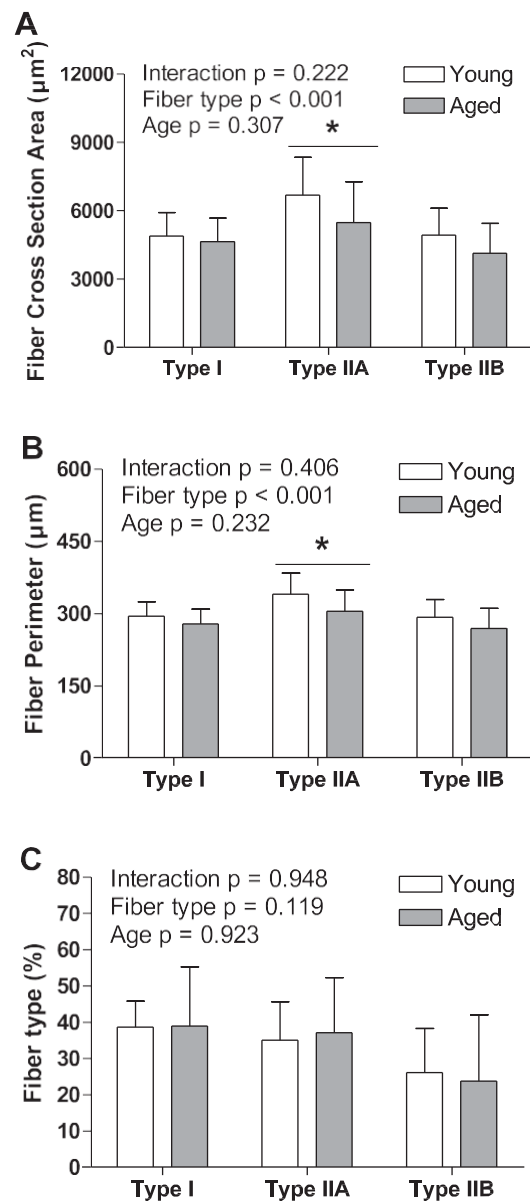


Fig. 1. Fiber cross-sectional area (A), fiber perimeter (B), and fiber type percent (C) for type I, IIA, and IIB fibers in young and aged men. Values are means (SD); $n = 6$ for young and 7 for aged. *Significantly different from type IIB fibers, $P < 0.05$.

Statistical treatment. For muscle fiber characteristics, mRNA, and protein, a two-way mixed-plot factorial analysis of variance (age X fiber type or age X rest/exercise) was used. Following a significant *F* ratio, a Bonferroni post hoc analysis was used. Unpaired Student's *t*-tests were used to compare differences in all other variables between young men and aged men. Multiple linear regression was performed to identify relationships between variables. There was inadequate sample for muscle morphology in two young men. Significance was established at $P < 0.05$ for all statistical sets, and data reported are means (SD).

RESULTS

Subject characteristics. Aged men were ~45 yr older than young men (Table 1). Aged men had greater body mass and a higher percentage of body fat. As anticipated, aged men demonstrated a significantly lower $\dot{V}O_{2\max}$, even when expressed per kilogram of fat-free mass.

Muscle morphology. There were no statistically significant differences in FCSA, FP, or fiber-type percent between young men and aged men (Fig. 1). FCSA and FP were greater in type IIA fibers compared with IIB fibers.

CC and C/F_i of type IIA fibers were greater than CC and C/F_i of type I or IIB fibers, respectively (Fig. 2). In aged men compared with young men, CC, C/F_i, and CFPE were lower regardless of fiber type. There was no difference in SF between groups (data not shown). A significant relationship was identified between $\dot{V}O_{2\max}$ and type I CFPE in aged but not young men (Fig. 3).

VEGF and VEGF receptor expression. In Fig. 4, mRNA at rest and 4 h postexercise for VEGF, KDR, and Flt-1 are shown normalized to 18S rRNA. Muscle VEGF mRNA was lower in aged men regardless of rest/exercise and exercise increased muscle VEGF mRNA independent of age group. Systemic exercise increased muscle KDR mRNA similarly between young men and aged men, whereas no effect of acute exercise or age was observed for muscle Flt-1 mRNA.

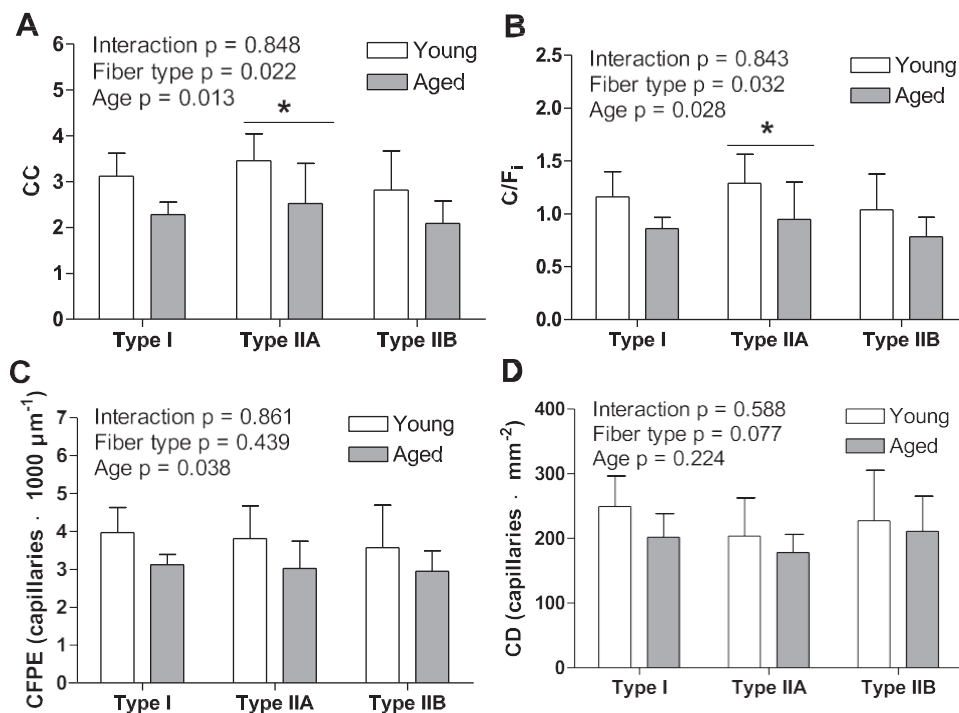
Muscle VEGF (A), KDR (B), and Flt-1 (C) proteins at rest and 4 h postexercise are shown in Fig. 5. Muscle VEGF protein was lower in aged men regardless of rest/exercise, and it was increased by exercise independent of age. There was no difference in muscle KDR or Flt-1 protein between groups or rest/exercise.

DISCUSSION

The principal findings of the present study from the vastus lateralis muscle of men are as follows: 1) CC, C/F_i, and CFPE are lower in aged compared with young regardless of fiber type; 2) VEGF mRNA and protein are lower in aged compared with young; and 3) VEGF protein is increased by acute exercise. To our knowledge, these are the first results demonstrating lower CC and CFPE of all three muscle fiber types in aged compared with young men, lower muscle VEGF expression in aged compared with young men, and an increase in muscle VEGF protein after a single acute exercise bout.

Aging and skeletal muscle capillarization. There are several different measures of muscle capillarization. In the present report, we have performed different measurements in an effort to understand whether absolute capillary numbers (CC and C/F_i) and relative measures of capillarization (CD and CFPE) are different between young and aged sedentary men. In terms of absolute capillary numbers, muscle CC and C/F_i were ~25% lower in aged compared with young men independent of fiber type. Proctor et al. (30) reported no difference in CC surrounding type I fibers (9%) but statistically lower CC of both IIA (23%) and IIB (30%) fibers in aged compared with young men. The difference between the present findings and those of Proctor et al. likely results from differences in the populations studied. The present report analyzed only sedentary young and aged men, whereas Proctor et al. concurrently analyzed groups of sedentary and trained young and aged men. As a result, low power may have prevented finding a statistical

Fig. 2. Capillary contacts (CC; A), individual capillary-to-fiber ratio (C/F_i; B), capillary-to-fiber perimeter exchange index (CFPE; C), and capillary density (CD; D) in young and aged men. CC, C/F_i, and CFPE were lower in aged compared with young men independent of fiber type. Values are means (SD); $n = 6$ for young and 7 for aged. *Significantly different from type I and IIB fibers, $P < 0.05$.



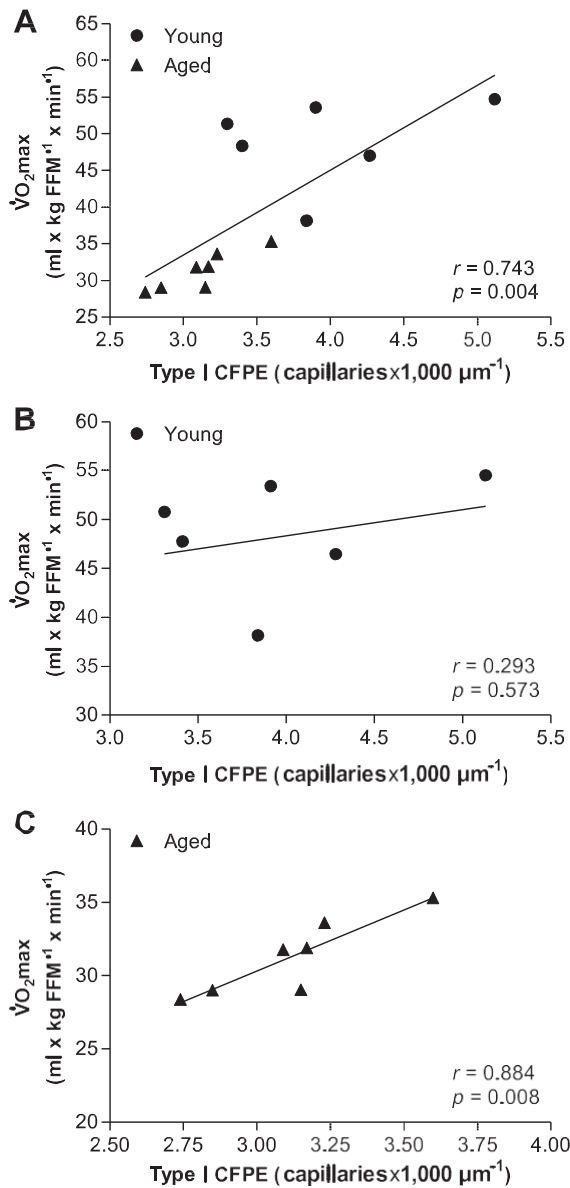


Fig. 3. Linear regression between maximal oxygen consumption ($\dot{V}O_{2\max}$) [per kg fat-free mass (FFM)] and type I CFPE for all subjects (A), young men only (B), and aged men only (C). $\dot{V}O_{2\max}$ is significantly related to CFPE in aged men.

difference in type I CC of the sedentary men (18% lower in aged sedentary men but similar between young and aged trained men) (30).

An interesting observation with respect to habitual exercise training is that training increases the number of capillaries surrounding type I, IIA, and IIB muscle fibers regardless of age (30). However, although exercise training may prevent the age-associated loss of muscle capillaries surrounding type I fibers, it does not prevent the loss of capillaries surrounding either type IIA or IIB fibers, suggesting that aging does lower angiogenic potential of type II muscle fibers (30). In elderly men, endurance exercise training promotes a greater relative increase in microvessel tortuosity than the increase in the number of capillaries surrounding muscle fibers (4). The greater relative increase in microvessel tortuosity in elderly men may be different from the response observed in young

muscle (28) and may represent a compensatory mechanism with aging to improve muscle diffusive capacity (4).

Because aging results in the loss of capillaries, it should be questioned whether the magnitude of the loss in capillaries is similar to the magnitude of the loss in FCSA and FP such that relative measures of muscle capillarization are unchanged with aging. In the present report, CD was similar between young and aged men, whereas CFPE was lower. Results from previous reports are equivocal, with lower CD (5) and the maintenance of CD (27, 30) in aged compared with young men. To our knowledge, the present report is the first to demonstrate lower CFPE in aged men, suggesting that overall capillary loss is greater than the loss in muscle fiber perimeter, which would have detrimental effects on muscle diffusive capacity. Current evidence suggests that the size of the capillary-to-fiber inter-

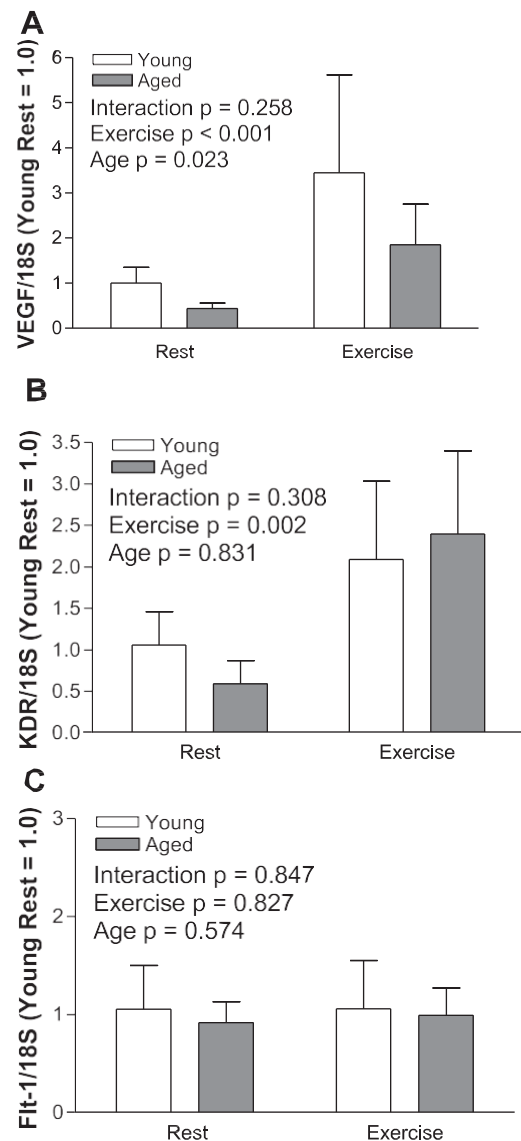


Fig. 4. Vascular endothelial growth factor (VEGF; A), KDR (B), and Flt-1 (C) mRNA in skeletal muscle of young and aged men at rest and 4 h postexercise. Young rest data were normalized to 1.0. All other data were normalized to young + rest to allow for comparisons. Exercise increased VEGF and KDR mRNA independent of group. VEGF mRNA was lower in aged men independent of rest/exercise. Values are means (SD); $n = 8$ for young and 7 for aged.

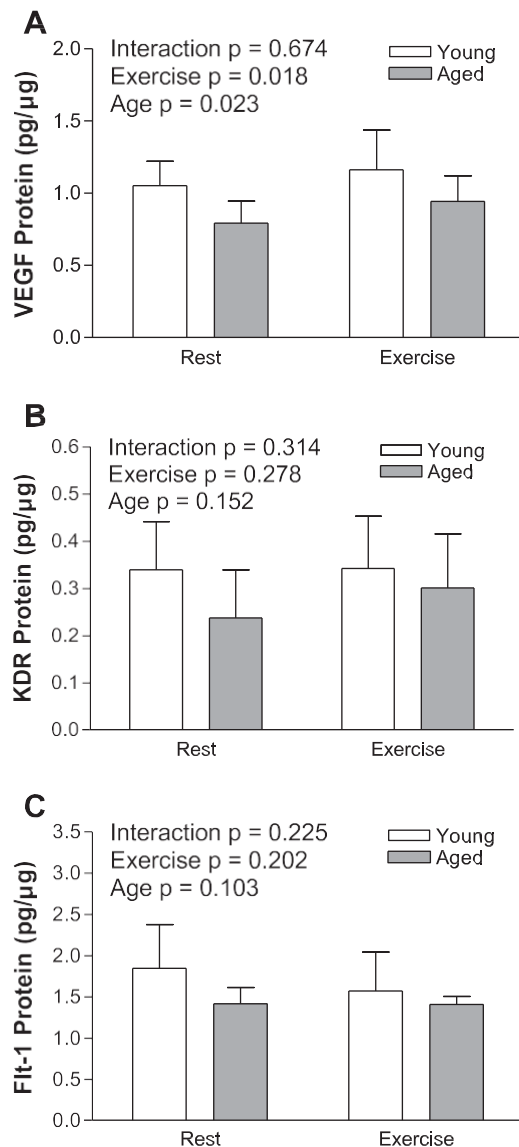


Fig. 5. Skeletal muscle protein for VEGF (A), KDR (B), and Flt-1 (C) at rest and 4 h postexercise. Exercise increased VEGF protein independent of group. VEGF protein was lower in aged men independent of rest/exercise. KDR and Flt-1 were unaffected by age or exercise. Values are means (SD); $n = 8$ for young and 7 for aged.

face is a major determinant of O_2 flux (19, 24). CFPE is an estimate of the capillary-to-fiber interface (21). Consistent with this, we observed that type I CFPE was significantly related to $\dot{V}O_{2\max}$ in aged men.

In our previous report, we observed that capillary contacts of type II, but not type I, fibers were lower in aged compared with young women (6). In an attempt to identify whether age-associated changes in muscle are different between men and women, we measured muscle fiber types and capillarization using the double-stain and myosin ATPase methods employed in the present report where possible in the women from our previous study to make direct comparisons between young and aged men and women (6). Six young and six aged women had sufficient sample remaining to perform the double stain and myosin ATPase stain. When young and aged men and women were analyzed by a two-way analysis of variance for each

individual variable, there were no age \times gender interactions identified ($P > 0.200$) except for a trend in type IIB CD where the interaction effect was $P = 0.095$. As a result, we analyzed the women separately by performing the same two-way analysis of variance (age \times fiber type) as performed in the men. Aged women demonstrated smaller FCSA of type IIA and IIB fibers and smaller FP of type IIB fibers compared with young women (Fig. 6), which is consistent with the type II fiber atrophy observed in these women using the Rosenblatt stain but is in contrast to our results in aged compared with young men. Similar to differences observed between aged and young men, muscle CC, C/F_i, and CFPE were lower and CD was not different in aged compared with young women (Fig. 7). These results clearly demonstrate that muscle capillarization is lower in aged compared with young men and women whether muscle

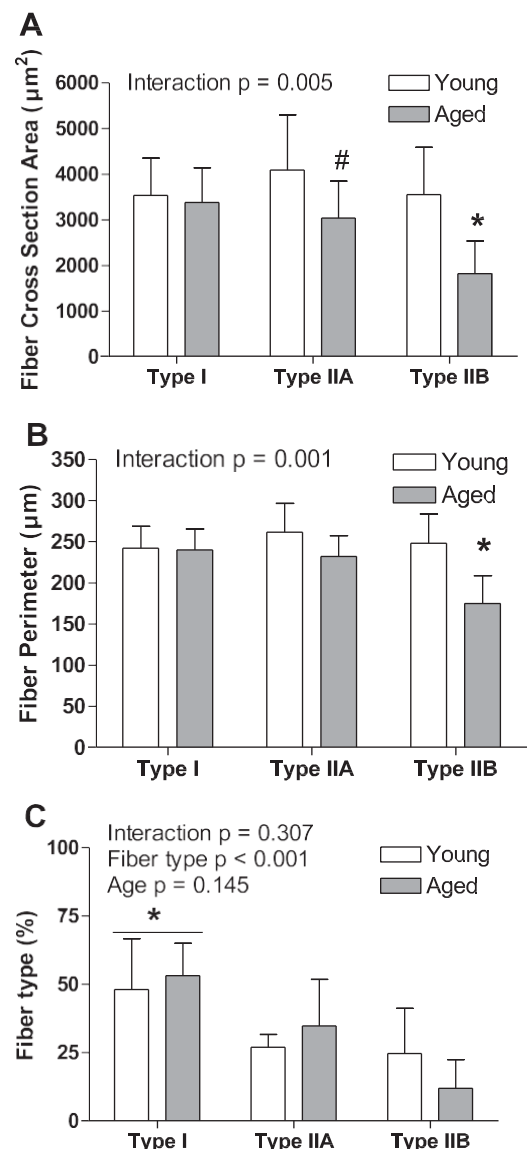


Fig. 6. Fiber cross-sectional area (A), fiber perimeter (B), and fiber type percent (C) for type I, IIA, and IIB fibers from selected young and aged women from Croley et al. (6) using the myosin ATPase stain. Values are means (SD); $n = 6$ for young and aged. *Significantly different from all other values, $P < 0.05$. #Significantly different from young type IIA, $P < 0.05$.

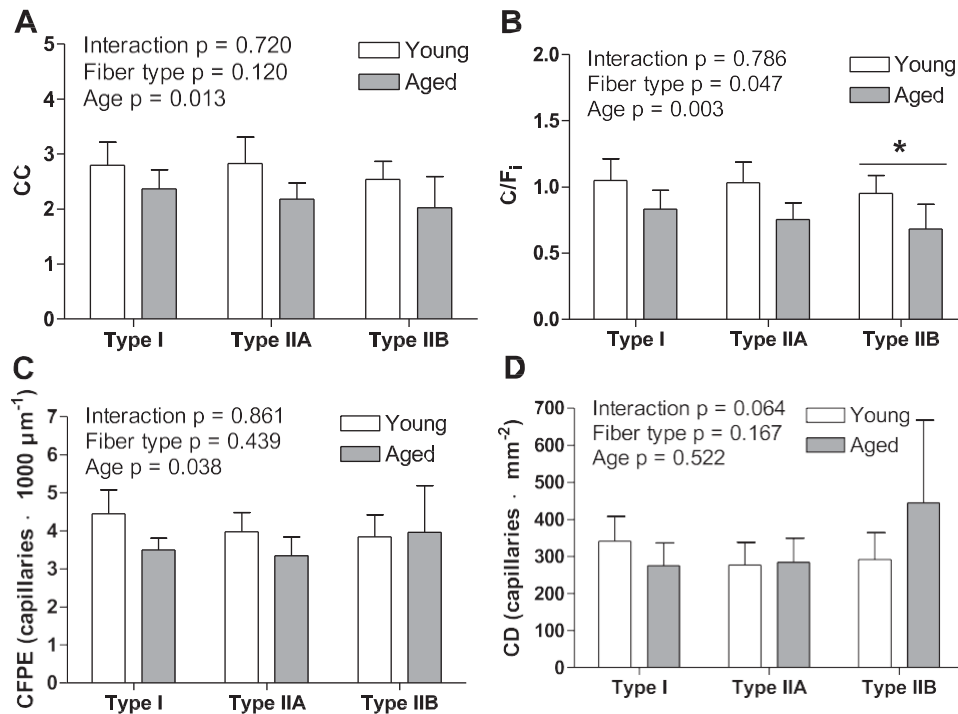


Fig. 7. CC (A), C/F_i (B), CFPE (C), and CD (D) from selected young and aged women from Croley et al. (6) using the double stain. CC, C/F_i, and CFPE were lower in aged compared with young men independent of fiber type. Values are means (SD); $n = 6$ for young and aged. *Significantly different from type I and IIA fibers, $P < 0.05$.

capillarization is reported by either absolute (CC and C/F_i) or relative (CFPE) measurements.

Aging, VEGF, and VEGF receptor expression. In the present report, muscle VEGF mRNA and protein were lower in aged compared with young men both at rest and postexercise. Recently, we observed that resting muscle VEGF protein was lower in aged compared with young women, whereas resting muscle VEGF mRNA was similar between groups (6). From our present and previous work (6), it is clear that muscle VEGF protein is lower in aged men and women. We are unaware of any report of an age-related gender-based difference in VEGF mRNA in any tissue.

It is well established that acute exercise can increase VEGF mRNA (13, 14, 16, 32); however, this response is lower in aged compared with young women (6). The present finding of lower VEGF mRNA after acute aerobic exercise in men is consistent with our previous finding in women (6), but it is inconsistent with a similar VEGF mRNA response to acute resistance exercise in aged compared with young men (21). Jozsi et al. (21) reported a similar 2.7-fold resistance exercise-induced increase in VEGF mRNA in young and aged men. As speculated previously (6), the difference in our data and that of Jozsi et al. most likely reflects different intracellular signaling events between aerobic and resistance exercise. However, it should be noted that the data reported by Jozsi et al. reflect fold changes and not absolute levels of VEGF mRNA, which may reflect less muscle VEGF mRNA in aged men.

In attempt to identify whether the age-associated differences in resting and exercise-induced muscle VEGF mRNA between the present findings and our previous report in women were the result of different techniques for measuring VEGF mRNA (Northern blot vs. real-time PCR), we measured VEGF mRNA using real-time PCR where possible from women in our previous study (6). Three young and three aged women had sufficient sample remaining to perform real-time PCR (Fig. 8).

Although the relative increase in muscle VEGF mRNA with acute exercise measured using real-time PCR was approximately half as great as previously observed using Northern blot, our previous results of similar resting muscle VEGF mRNA and an attenuated muscle VEGF mRNA response to acute exercise in aged compared with young women are consistent between techniques. Therefore, the greatest difference in VEGF mRNA observed with aging between men and women is that aged men demonstrate lower resting muscle VEGF compared with young men, whereas VEGF mRNA postexercise in aged men and women is very similar compared with their respective young counterparts.

In addition to an increase in muscle VEGF mRNA, we also observed a 15% increase in muscle VEGF protein in response to a single acute exercise bout. Previously, our laboratory

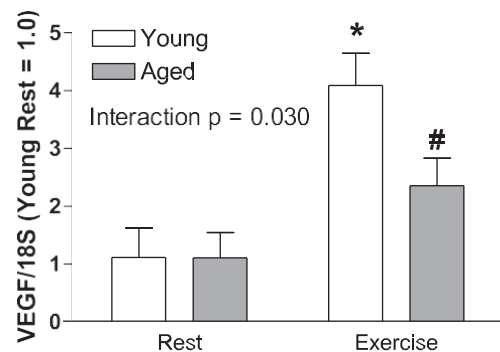


Fig. 8. VEGF mRNA in skeletal muscle of young and aged women at rest and 4 h postexercise from our previous report (6). Young rest data were normalized to 1.0. All other data were normalized to young + rest to allow for comparisons. VEGF was significantly increased by exercise 4.1-fold in young women. In aged women, the VEGF mRNA response to acute exercise was significantly lower (2.3-fold increase). Values are means (SD); $n = 3$ for young and aged. *Significantly different from all other conditions, $P < 0.05$. #Significantly different from young + rest and aged + rest, $P < 0.05$.

reported a decrease in muscle VEGF protein immediately after the conclusion of acute exercise, with a return to baseline at 2 and 4 h postexercise (13). In additional reports, our laboratory did not observe an increase in muscle VEGF protein at either 2 h post-aerobic exercise in lean and obese young men (14) or 4 h postexercise in young and aged women (6). In these individuals, exercise increased muscle VEGF protein in ~60% of cases (6, 14). In the present report, muscle VEGF protein was increased in 11 of 15 (73%) individuals, which may explain our finding of a significant increase in muscle VEGF protein with acute exercise in the present report.

In similar fashion to our comparisons of muscle morphometry and VEGF mRNA between young and aged men and women, we wished to compare VEGF protein levels between the present report and our previous findings (6). However, VEGF protein is ~50% lower in samples homogenized using a Dounce compared with a Polytron homogenizer (unpublished observations), making direct comparisons of VEGF impossible between the two data sets. We have recently shown that, when isolated in a similar manner, VEGF protein is ~20% lower in young women compared with young men and that muscle capillarization is strongly related to muscle VEGF protein (6). Therefore, muscle VEGF is greatest in young men and lowest in aged women with muscle VEGF in young women and aged men in between these groups. It must be highlighted that muscle VEGF protein was significantly lower (~25%) in aged compared young men, which is consistent with our previous report of lower (~30%) muscle VEGF in aged compared with young women (6).

Aging lowers VEGF mRNA expression in breast tissue (15) and fibroblasts (23) in humans, lowers VEGF protein in the kidney (22) and carotid bodies (7) in rats, and lowers hypoxia-induced VEGF mRNA and protein expression in rabbit and mouse skeletal muscle and in rabbit smooth muscle cells (33, 34). The reduction in hypoxia-induced VEGF expression in aged smooth muscle cells results from lower binding activity of hypoxia-inducible factor-1 (34), a well-known transcriptional regulator of VEGF. It has been proposed that hypoxia regulates in part exercise-induced increases in muscle VEGF expression (37), and recent data suggest that acute exercise with restricted blood flow increases muscle hypoxia-inducible factor-1 binding activity in humans (2). Whether lower skeletal muscle VEGF expression in aged compared with young humans is due to lower hypoxia signaling remains to be elucidated.

There was no effect of age on either KDR or Flt-1 mRNA or protein. Given that capillary density is similar between young and aged, this result is not surprising and suggests that KDR and Flt-1 content within endothelial cells is similar in young and aged men and is consistent with findings in young and aged women (6). KDR mRNA was increased by acute exercise, whereas Flt-1 mRNA was not. Our laboratory (6, 13, 14) and others (17) have previously observed increases in both KDR and Flt-1 mRNA in response to acute exercise in humans. Consistent with this, several reports have shown an increase in Flt-1 mRNA with acute exercise in rats (9–12, 26), but an increase in Flt-1 mRNA with acute exercise has not always been observed in rats (27).

In summary, we have demonstrated that muscle capillarization is lower and that muscle VEGF expression is lower in aged compared with young men both at rest and after acute aerobic exercise. These results are consistent with lower muscle cap-

illarization and muscle VEGF expression recently reported in aged compared with young women (6). Our data suggest that lower VEGF expression may play an important role in the lower muscle capillarization observed in aged compared with young men.

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GRANTS

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